

Release of Tryptophan and Serotonin into the Portal Vein of the Isolated Perfused Rat Small Intestine*

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Summary. To investigate the release of serotonin from intestinal enterochromaffin cells, we used an in vitro technique which allows studies excluding overlapping influences from outside the gut. The entire small intestine of rats fed a standard or tryptophan-enriched (3% of total) diet was totally isolated by ligatures with the exception of the superior mesenteric artery and portal vein that supply and drain the intestine. Simultaneously to the vascular perfusion (Krebs-Ringer bicarbonate buffer, 0.4% human albumin, 5 mM glucose, 0.6 mM glutamine) the gut lumen was infused (buffer or 0.1 N HCL). Acidification of the gut lumen resulted in an increment of venously released tryptophan and serotonin. After feeding tryptophan-enriched food the release of tryptophan was increased. However, the total amount of released serotonin after tryptophan diet did not differ as compared to that after standard diet. Addition of a monoamino-oxidase inhibitor (pargyline) to the arterial perfusate enhanced the released amount of serotonin 3-fold in the portal venous effluent (at a concentration of 1 mM but not 0.1 mM). Recovery studies done by arterial infusions of serotonin (1 μ M, 10 μ M) and evaluation of the amounts venously released revealed a high loss of infused serotonin (40%–70%). Our data suggest gut-born serotonin to more likely play a paracrine role than a role as a classical hormone.

Key words: Serotonin – Tryptophan – Small intestine – Rat – Pargyline

Introduction

The gastrointestinal tract represents the major source of serotonin in mammals so that circulating serotonin is derived predominantly from mucosal enterochromo-

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maffin cells [2, 8, 10, 13]. Little is known about the control of serotonin release from such cells or the physiologic functions of serotonin subsequent to its release [10, 22]. The evidence from previous *in vivo* studies was largely indirect and circumstantial.

To investigate the release of serotonin from intestinal enterochromaffin cells, we used an *in vitro* technique which permitted the study of the whole intact small intestine of rats under almost physiologic conditions with access to the gut lumen, arterial and venous circulation. This allowed studies excluding overlapping influences from outside the gut. By use of this approach the release of serotonin and tryptophan after luminal acidification in rats of a standard or tryptophan-enriched diet was studied.

Material and Methods

Animals and Diets

In one group of female Wistar rats (220–240 g b.wt.) fed a standard diet (Altromin 1324, Lage/Lippe, FRG) the release of tryptophan and serotonin was studied after acidification of the lumen of the isolated perfused small intestine. In a second experimental approach rats (180–220 g b.wt.) were pair-fed a standard or a tryptophan-enriched (L-tryptophan added as 3% of total diet) food over 10 days. These rats were used for perfusion experiments ($n = 15$) or tissue extractions ($n = 12$). The day before perfusion studies were undertaken the animals were fasted over 12 h and included into the respective experiments. All rats were anesthetized *i.p.* with pentobarbitone (45 mg/kg b.wt.).

Perfusion of the Small Intestine

The preparation of the isolated, perfused small bowel was performed according to Cobb et al. [4]. Oxygenated perfusion fluid entered the gut preparation by way of the aorta through the A. mesenterica superior. A cannula was put into the portal vein and tied for subsequent collection of portal venous effluent. A tube was placed into the proximal duodenum through an incision into the stomach for feeding of the gut lumen. Another tube was inserted into the cecum for the drainage of feeding fluid. The entire small intestine was vascularly perfused at a constant rate (4 ml/min) with Krebs-Ringer bicarbonate buffer containing 0.4% human albumin, 5 mM glucose, and 0.6 mM glutamine, 37°C, gassed with 95% O₂ and 5% CO₂. Changes in pressure measured by a Statham pressure transducer connected to a recorder reflected changes in vascular resistance. The gut lumen was fed by way of the tube placed in the duodenum. Feeding rate was 0.38 ml/min. The feeding fluid consisted of Krebs-Ringer bicarbonate buffer or HCl (0.1 N) as was suggested previously [23]. One perfusion experiment lasted for 30 min. Over an initial period of 10 min KRB-buffer was introduced into the gut lumen. Thereafter, acidification of the lumen was performed by HCl infusion over 10 min followed by KRB buffer administered over the last 10 min of perfusion time. The venous effluent of the perfused intestine was collected at 2-min intervals and monitored for tryptophan and serotonin.

Serotonin and Tryptophan Measurements

In the venously collected samples serotonin and tryptophan concentrations were measured by reverse-phase HPLC using a Waters Radial PAK μ -Bondapak C18 cartridge and a Kontron SFM-23 fluorimeter [24]. The small intestines were extracted as described previously [21, 25].

Statistic

Analysis was performed by the *U*-test of Wilcoxon, Mann and Whitney.

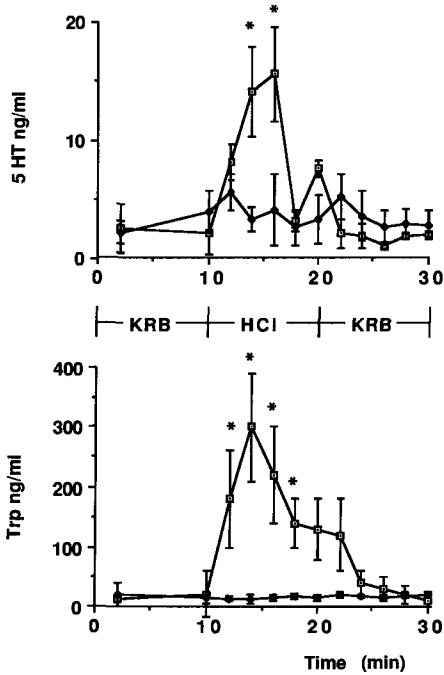


Fig. 1. Release of tryptophan (lower panel) and serotonin (upper panel) from the perfused small intestine after acidification of the gut lumen. Given are means \pm SEM of $n = 5$ experiments/group. Asterisks symbolize significance ($p < 0.05$) of the differences between controls (sole KRB-buffer perfusion into the gut lumen; closed circles) or after acidification (open circles)

Table 1.

a Tryptophan and serotonin tissue concentrations in the rat small intestine after administration of a tryptophan-enriched diet

	Tryptophan (ng/g wet weight)	Serotonin (ng/g wet weight)
Controls ($n = 6$)	2100 (400)	3291(1343)
Tryptophan diet ($n = 6$)	6200 (694) ²	11430 (3033) ²

b Summarized release of tryptophan and serotonin from the perfused intestine; effect of tryptophan-enriched diet and pargyline (1 mM)

	Tryptophan (ng/30 min)	Serotonin (ng/30 min)
Standard diet ($n = 5$)	1152 (273)	483 (94)
Tryptophan diet ($n = 5$)	2634 (202) ²	583 (209)
Tryptophan diet + pargyline ($n = 5$)	2340 (291) ²	1399 (174) ²

² $P \pm 0.05$; given are means (SEM)

Results

Figure 1 demonstrates the release of tryptophan and serotonin from the perfused intestine after acidification of the gut lumen of rats on standard diet. The release of tryptophan and serotonin was significantly increased in response to the onset of luminal acid infusion. This effect was reversed again when Krebs-Ringer buffer was infused instead of acid during the last 10 min of the experiment. Feeding

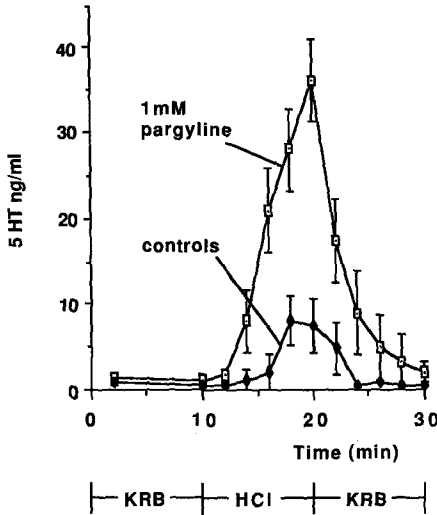


Fig. 2. Effect of 1 mM pargyline upon serotonin release from the small intestine after tryptophan-rich diet ($n = 5$; open circles). Controls ($n = 5$; closed circles) received tryptophan diets, but had no pargyline in the vascularly perfused buffer medium. Given are means \pm SEM. For the summarized secretion rates of experiments see Table 1

tryptophan-enriched food increased the tryptophan and serotonin tissue levels (Table 1) in accordance to earlier reported findings [11]. However, the total amounts of released serotonin after tryptophan-enriched food were not significantly different from those released after administration of standard diet. In contrast to this, the released amount of tryptophan after tryptophan-enriched diet was above controls (Table 1).

Addition of the monoamino-oxidase inhibitor pargyline to the perfusate (1 mM) enhanced the released serotonin amount from intestines of tryptophan-fed rats 3-fold as compared to controls perfused with medium lacking the inhibitor (Fig. 2 and Table 1). Addition of 0.1 mM pargyline to the perfusate had no effect (data not shown).

Arterial infusion of 1 ($n = 3$) or 100 μ M ($n = 1$) serotonin drastically increased the vascular resistance measured arterially (mean Δ P was 40 mmHG). These recovery studies performed in single experiments by comparing the venously released amounts of serotonin with those arterially infused revealed a high loss of serotonin (40%–70% of infused serotonin) during the vascular passage through the intestinal vascular system. These experiments allowed no precise quantitative analysis, since the observed drastic increase of vascular resistance was accompanied by a development of a significant edema and a progressive loss of vascularly infused buffer volume into the gut lumen.

Discussion

Previous investigators have predominantly relied on *in vivo* approaches for studying the release of serotonin from the gut [17]. However, those studies were hampered by methodologic problems in the measurement of circulating concentrations of serotonin [17, 18]. First, the proper handling of blood samples is a crucial problem since more than 90% of circulating serotonin is associated with

platelets and the occurrence of hemolysis alter serotonin measurements grossly. Secondly, when released from intestinal cells most of the serotonin undergoes prompt degradation during its passage through liver and lung [7]. Therefore, serotonin measurements in peripheral blood allow only limited conclusions on the amount of the amine released from gut cells into the portal vein.

The *in vitro* vascularly perfused small intestine offers advantages as an approach to study the release of serotonin from intestinal cells. The use of buffered, oxygenated salt solutions omits analytical problems otherwise possibly induced by destruction of platelets or hemolysis. The preparation of the isolated rat intestine was demonstrated previously to offer a useful tool in studying the release and catabolism of hormones and other biologic compounds [3, 6, 16, 26, 27]. Perfusions with buffered salt solutions lasting for longer than 30 min resulted in only a poor viability of the perfused gut tissue [5, 12]. An improved survival of the *in vitro* perfused gut was reported before by others using vascularly perfused buffers supplemented with blood cells [6, 15, 20]. However, the need to avoid blood constituents in our experiments is evident. Therefore, we limited our perfusion time to 30 min. Our experiments demonstrated that in accordance with *in vivo* experiments [8, 23] luminal acidification of the gut results in a vascular release of serotonin and tryptophan. The flow rate in the portal vein remained constantly during acidification. This was controlled by an evaluation of the venously released volumes of effluents per time interval which remained unaltered. Furthermore, the vascular resistance measured arterially increased only slightly. Therefore, a change of flow rates as consequence of luminal acidification which could possibly influence the serotonin concentrations measured in the effluents was not observed. We have not studied the morphological appearance of the gut mucosa after perfusion with hydrochloric acid. This was done earlier by Resnick and Gray [23]. They reported a reduction of the serotonin content of the upper small intestine of rats and a degranulation of argentaffin cells by perfusion of the gut lumen with 0.1 N hydrochloric acid. In these experiments histological studies of the small intestine revealed no gross morphological abnormality after intraluminal HCl. Released from gut cells rapid degradation of the biogenic amines occurs. This can be ascribed to amine uptake mechanisms [7] and inactivating enzymatic activities [7] in the gut. The resulting serotonin inactivating effect has a big capacity, since even administration of tryptophan-enriched food did not enhance the vascularly released serotonin amount, and an arterial infusion of serotonin resulted in high losses of the amine when recovery measurements were performed in the venous effluents. Our data obtained by use of a MAO-inhibitor (pargyline) support the concept of a major role for monoamino-oxidase activities in the degradation of vascular released serotonin in rats.

In summary, the present data make a role for intestinal serotonin as a "classical" circulating hormone rather unlikely. However, it is possible that the well known uptake of serotonin into platelets [7] leads to physiological relevant survival of intestinal serotonin in the circulation. Otherwise, a paracrine or "local" role for the amine, e.g. in the regulation of local blood flow, could be of importance. Recent evidence for a physiologic role of intraluminally released serotonin emphasize the possible importance of serotonin as local modulator of intestinal functions [9, 14, 19].

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